In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 8, lines 12-17 and replace it with the following paragraph:

As used herein, a "glycine-rich linker" comprises a peptide sequence with two or more glycine residues or a peptide sequence with alternating glycine and serine residues, in particular the amino acid sequences Gly-Gly, Gly-Ser-Gly, and Gly-Gly-Ser-Gly-Gly **(SEQ ID NO: 43)**. With regard to glycine-rich linkers reference is made to Witchlow M. et al., "An improved linker for single-chain Fv with reduced aggregation and enhanced proteolytic stability", (1993) *Prot. Engineering*, **6**:989-995.

Please delete the paragraph on page 15, lines 8-29 and replace it with the following paragraph:

In another preferred embodiment the modified calcium-binding polypeptide of the invention further comprises a localization signal, in particular a nuclear localization sequence, a nuclear export sequence, an endoplasmic reticulum localization sequence, a peroxisome localization sequence, a mitochondrial input sequence, a mitochondrial localization sequence, a cell membrane targeting sequence, and most preferably a cell membrane targeting sequence mediating localization to pre- or postsynaptic structures. It has been found that a particular advantage of the modified calcium-binding polypeptides of the invention is that they function in the context of subcellular environments where prior art calcium sensor have failed to work or have shown poor performance. The calcium sensors of the present invention are therefore particularly powerful when targeted to specific subcellular structures, like organelles or functionally distinct regions of the cell-like lamellipodia or filophodes or axons and dendrites in the case of neuronal cells. Such subcellular targeting can be accomplished with the help of particular targeting sequences. Localization to the endoplasmic reticulum can be achieved by fusing the signal peptide of calreticulin, MLLSVPLLLGLLGLAAAD **(SEQ ID NO: 44)** to the N-terminus of a fusion polypeptide and the sequence KDEL **(SEQ ID NO: 45)** as an ER retention motive to

the C-terminus of a fusion polypeptide (discussed in Kendal et al. "Targeting aequorin to the endoplasmic reticulum of living cells." *Biochem. Biophys. Res. Commun.* **189**:1008-1016, (1992)). Nuclear localization can be achieved, for example, by incorporating the bipartite NLS from nucleoplasmin in an accessible region of the fusion polypeptide or, alternatively, the NLS from SV 40 large T-antigen. Most conveniently, those sequences are placed either at the N- or the C-terminus of the fusion polypeptide.

Please delete the paragraphs on page 16, line 20 to page 17, line 2, and replace them with the following paragraphs:

For targeting to the inner leaflet of the cell membrane, the first 20 amino terminal amino acids of GAP-43 (growth associated protein) are useful, i.e. the sequence MLCCMRRTKQVEKNDEDQKI **(SEQ ID NO: 46)**. Alternatively, membrane targeting can be achieved by fusing the 20 most C-terminal amino acids of C-Ha-Ras to the C-terminus of a fusion polypeptide. These amino acids are KLNPPDESGTGCMSCKCVLS **(SEQ ID NO: 47)**. (For reference see Moryoshi K. et al. (1996) *Neuron,* **16**:255-260.)

Targeting to postsynaptic sites can be achieved by fusing the C-terminal PDZ-binding domain of the NMDA-receptor 2B subunit to the C-terminus of a fusion polypeptide. The sequence is VYEKLSSIESDV **(SEQ ID NO: 48)**. Alternatively, the PDZ-binding domain of the inwardly rectifying potassium channel KIR 2.3 can be used as a localization when added to the C-terminus. The sequence is MQAATLPLDNISYRRESAI **(SEQ ID NO: 49)**. (For reference see Liedhammer M. et al. (1996) *J. Neurosci.*, **16**:2157-63, and Lemaout S. et al. (2001) *Proc. Natl. Acad. Sci. USA,* **98**:10475-10480.) Other PDZ-binding domains useful for localizing indicators can be found in Hung and Sheng (2001*) J. Biol. Chem.,* **277**:5699-5702.

Please delete the paragraph on page 24, line 18 to page 25, line 2 and replace it with the following paragraph:

Figure 2: Summary of basic constructs and evaluation of their function. csTnC, chicken skeletal muscle troponin C. csTnC-N90, the N-terminal lobe of chicken skeletal troponin C (amino acids 1-90). csTnC-EFn, the individual EF hands 1-4 of chicken skeletal muscle troponin C. csTnI, chicken skeletal muscle troponin I. csTnI 1-48, csTnI 95-133, csTnI 116-135, various short peptides derived from chicken skeletal muscle troponin I consisting of the indicated amino acid residues. csTnC-L15, truncated chicken skeletal muscle troponin C in which the N-terminal amino acid residues 1-14 are deleted, which makes the protein start at leucin 15. The whole indicator construct was named TN-L15 (SEQ ID NO. 1 and 2). csTnC-L15 D107A, csTnC-L15 carrying the mutation D107A. The whole indicator construct was named TN-L15 D107A (SEQ ID NO. 5, 6). csTnC-L15-N90, N-terminal lobe of chicken skeletal muscle troponin C consisting of amino acid residues 15-90. hcardTnC, human cardiac muscle troponin C. The whole indicator construct is referred to as TN-humTnC (SEQ ID NO. 3, 4). hcardTnC1-135, human cardiac muscle troponin C lacking the last EF hand domain. hcardTnC-L12, human cardiac muscle troponin C in which the N-terminal amino acid residues 1-11 are deleted, analogous to csTnC-L15. L, linker: either GG, GSG or GGSGG **(SEQ ID NO: 43)**.

Please delete the paragraphs on page 30, line 24 to page 31, line 26 and replace them with the following paragraphs:

To alter calcium affinitites of single EF-hands of chicken skeletal muscle troponin C, point mutations were introduced into the gene sequence by site-directed mutagenesis using the primer extension method (QuickChange, Stratagene). For protein expression in mammalian cells, an optimized Kozak consensus sequence (GCC GCC ACC ATG G **(SEQ ID NO: 50)**) was introduced by PCR at the 5' end of CFP; the entire indicator fragments obtained by BamHI/EcoRI restriction of the pRSETB constructs were then subcloned into the mammalian expression vector pcDNA3 (Invitrogen). Membrane targeting of indicator proteins was achieved by extending the indicator DNA sequences with a sequence encoding a membrane localization signal by PCR. In particular, the 20 amino acid sequence KLNPPDESGPGCMSCKCVLS **(SEQ ID NO: 51)** of the c-Ha-Ras membrane-anchoring signal was fused at the 3' end of the indicator sequences, and the 20

amino acid sequence MGCCMRRTKQVEKNDEDQKI **(SEQ ID NO: 52)** of the GAP43 membrane-anchoring signal was fused at the 5' end. See Moriyoshi K., et al., "Labeling Neural Cells Using Adenoviral Gene Transfer of Membrane-Targeted GFP." *Neuron* **16**, 255–260 (1996).

Fusions of TN-L15 (SEQ ID No: 1) or YC3.1 to Synaptobrevin were made by amplifying Synaptobrevin by PCR, thus introducing a Kpn1-Site within a GGTGGS **(SEQ ID NO: 53)** linker to its 5'-end. Simultanously, a Kpn1-site was introduced at the 3' end of csTnL-15 or YC3.1, respectively. The stop codon was thereby deleted. DNA fragments coding for thus modified Synaptobrevin and TN-L15 or YC3.1 were ligated together into an expression plasmid.

For the construction of the non-Aequoria victoria-FP indicator version Cop-L15-Phi, DNA sequences of Cop-Green (Copepoda-GFP ppluGFP2) and Phi-Yellow (Phialidium-YFP) were obtained by PCR from cDNA-containing plasmids (both Evrogen). The sense primer used for the amplification of the Cop-Green insert introduced a BamH1 restriction site and the Kozak sequence GCC GCC ACC ATG GCC **(SEQ ID NO: 54)** at the 5' end of the Cop-Green sequence, thereby adding the new amino acids Met and Gly to the N-terminus of the polypeptide chain. The antisense primer inserted a SphI restriction site at the 3' end of the Cop Green sequence and deleted the original stop codon. The Phi-Yellow insert was amplified with a primer pair that introduced a SacI site at its 5' end and a EcoRI site at its 3'end. For the creation of the indicator construct Cop-L15-Phi, a chicken skeletal muscle troponin C (csTnC-L15) fragment containing amino acids 15-163 with a SphI site at the 5' end and a SacI site at the 3' end was ligated together with the Cop-Green and Phi-Yellow inserts into the expression vector pRSETB (Invitrogen). This resulted in the fusion protein Cop-L15-Phi with the FRET donor Cop-Green at the N-terminus, csTnC-L15 as calcium binding domain in the middle, and Phi-Yellow as FRET acceptor at the C-terminus.